compared to the minor-mismatched pair. Conversely, when WT HSC and TCs were transplanted into myeloablated and non-myeloablated μMT mice, BC engraftment was readily established, making a direct anti-BC cytotoxicity unlikely as the sole cause of the BC inhibition. FACS analysis of the bone marrow revealed a shift of the developmental BC stages of from mature to immature stages in B lymphopenic mice. While GVHD affected mice that received WT grafts displayed a lower proportion of IgM expressing BCs, recipients of μMT TCs showed a complete block in BC development with an absent switch to the expression of IgM. In conclusion, TCs from μMT mice can block BC maturation when transferred into WT mice, an observation which shows that mature TC are capable of interfering with BC regeneration post transplantation. This HCT model using WT and μMT B6 mice provides a powerful tool for studying the role of TC function in the process of donor BC development post-HCT.

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B LYMPHOCYTE RECONSTITUTION FOLLOWING ALLOGENEIC STEM CELL TRANSPLANTATION FOR HAEMATOLOGICAL DISORDERS: CORRELATION WITH PRE- AND POST-TRANSPLANT FACTORS

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We have performed a detailed analysis of the kinetics of B cell reconstitution in patients who have undergone allogeneic stem cell transplantation for haematological disorders. Whole blood samples from 76 patients were analysed using monoclonal antibodies directed against CD19, CD27 and IgD by multicolour flow cytometry. Total (CD19+), naive (IgD+CD27-), IgD memory (IgD+CD27+) and class switched memory (IgD-CD27+) B cell populations were identified. Indications for transplantation were haematological malignancy (n = 74) and aplastic anaemia (n = 2). The stem cell source was from an unrelated donor in 29 cases or related/sibling donor in 47 cases. 39 patients received reduced intensity and 37 full intensity transplant conditioning regimens. In vivo T-cell depletion with Campath 1H was used for all patients with a matched unrelated donor and those receiving reduced intensity conditioning. The majority of patients (n = 57) have retained a predominantly naive B cell phenotype at 2 to 48 months (median 18.7 months). 7 patients have a normal percentage of memory B lymphocytes with a low percentage of class-switched B cells. 7 patients currently have a normal percentage of class-switched B cells. 8 patients achieved normal class-switched B cell numbers but returned to a naive B cell phenotype. In one patient this preceded relapse of underlying leukaemia and in two others was consequent on treatment with chemotherapy or corticosteroid. Only one patient who required treatment with additional immunosuppressive therapy, post transplant, subsequently achieved a normal percentage of class-switched B cells. The use of Campath 1H, stem cell source (marrow or peripheral blood), donor type (unrelated or related), use of full or reduced intensity conditioning or the administration of donor lymphocyte infusions post-transplant were not predictive of development of a normal percentage of class-switched B lymphocytes. B cell immune reconstitution phenotype does not appear to correlate with the incidence of chronic GvHD or in this cohort of patients with current disease status. The timing of development of a normal percentage of class-switched B cells cannot be reliably predicted in this patient population.

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MINIMAL RESIDUAL DISEASE AND CHIMERISM IN CML PATIENTS RECEIVING ALLOGENEIC TRANSPLANTS AFTER MYELOABLATIVE OR REDUCED CONDITIONING

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It is not known whether reduced conditioning is associated with prolonged period of detectable disease after SCT in patients with CML. We have therefore compared BCR-ABL transcript levels in patients receiving a conventional myeloablative conditioning with those receiving reduced conditioning. We also made chimerism studies in both patient groups.

Myeloablative Conditioning (MYC): 31 patients, 19 matched unrelated donors (MUD) and 12 sibling donors (Sib). Median age was 42 years (10–61). Conditioning with Bu+Cy (n = 31). Twenty of the MUD patients were also given ATG. Seven patients have died (6 GVHD, 1 Relapse). Median follow-up time: 56 months (10–99). **Reduced Intensity Conditioning (RIC):** 24 patients, 13 MUD and 11 Sib. Median age was 55 years (11–64). Conditioning with Flu+Bu+ATG. Seven patients have died (3 Relapses, 1 GVHD, 3 other reasons). Median follow-up time: 43 months (18–100). **Results:** There was no statistical significant difference in overall survival and relapse free survival between both groups. 5/29 patients in the MYC group relapsed as compared to 7/23 patients in the RIC group. Only one patient in the RIC group rejected the graft. The incidence of severe acute GVHD was significantly higher in the MYC group (48%) as compared to the RIC group (13%), p = 0.004. Interestingly, the incidence of chronic GVHD was slightly higher in the RIC group, 51% *vs.* 37% (p = 0.11).

During the first 3 months, MRD levels in the RIC group was in median one log of magnitude higher than in the MYC group (p = 0.01). After that, no significant differences in the MRD incidence and MRD level were found between the groups.

Median time to complete donor T-cell chimerism was 34 days (14–285) in the MYC group as compared to 60 days (24–245) in the RIC group (p = 0.04). Also, complete myeloid cell engraftment was delayed in the RIC group, median 44 days (14–165) as compared to 25 days (14–270) in the MYC group (p = 0.02). **Conclusion:** Despite higher BCR-ABL levels during the early post-transplant period and higher incidence of mixed chimerism, nonmyeloablative transplantation for CML patients may induce molecular remission in the majority of the patients.

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TRANSCRIPTIONAL ANALYSIS OF CD4+ T CELLS FROM TOLERANT PATIENTS AND NORMAL CONTROLS. GENE EXPRESSION IN PATIENTS DIFFERS FROM NORMAL CONTROLS

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GVHD is a major complication of hematopoietic cells transplantation (HCT). Immune suppression therapy (IST) is administered for prophylaxis and treatment of GVHD. Chronic GVHD usually occurs within 3 to 24 months from HCT, and requires on average 24 months of IST before disease becomes quiescent and IST can be withdrawn. We have characterized gene expression changes in CD4+ T cells of HCT patients with resolved GVHD to see if distinct translational changes are associated with immunological tolerance. The study included 10 patients who had been off all IST for at least 4 months (median 9 months; range 4–38 months) without evidence of active cGVHD. Six achieved tolerance within the first year ("early" tolerance). Four developed cGVHD within the first year but were subsequently withdrawn from all IST between 6–38 months post-HCT ("late" tolerance). Blood samples from early tolerants were obtained between 12–29 (median 12) months, from the late tolerants were obtained between 38–52 (median 45) months post-HCT. CD4+ T cells were isolated from cryopreserved PBMC. RNA was hybridized on HumanRef-8 v2 BeadChips, scanned and normalized using BeadStudio (Illumina), gene expression in CD4+ T cells from the 10 tolerant patients was compared to gene expression in CD4+ T cells from 19 normal controls using a false positive discovery rate of <10. Expression levels differed for 266 genes in tolerant patients (124 upregulated and 142 downregulated) compared to normal controls; and 189 of these genes could be assigned to a functional class. Selected examples are: DNA repair (6 genes); cell cycle regulation (18 genes); RNA transcription (9 genes); protein translation (8 genes); ubiquitin cycle (6 genes); glycolysis and aerobic respiration pathways (5 genes); and lipid metabolism (3 genes). FAS gene showed relatively strong upregulation in all patients. Although the numbers of early and late